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Elemental Analysis of Human Cremains Using ICP-OES to Classify Legitimate and Contaminated Cremains*

ABSTRACT: The Tri-State Crematory Incident in Nobel, GA (February 2001) revealed limitations in traditional human cremated remains (cremains) analytical methodology. The goal of this study was to develop a method for effectively classifying questionable sets of cremains as legitimate or contaminated. Eighty-eight samples of known human cremains, concrete, mixtures of the two, and questionable sets of cremains were acid digested and analyzed for 21 elements by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Variable cluster and principle component analyses identified the seven elements (Sb, B, Li, Mn, Sr, Tl, and V) used to develop discriminant functions to classify questionable sets into two groups: cremains and concrete. The discriminant analysis shows that at the 0.90 probability level, mixtures of 50% or less human content were classified as concrete. Mixtures with 90% human content classified as cremains. Sixty percent and 75% human content mixtures remained in the questionable classification, but as the concentration of human increased in the mixture, the probability of assignment to the known cremains group increased. Most of the questionable human samples classified as cremains. This is a pilot study and cannot yet satisfy Daubert standards for courtroom admissibility, but it indicates that it is possible to determine the legitimacy of cremains using elemental analysis by ICP-OES coupled with multivariate statistical analysis.

KEYWORDS: forensic science, forensic anthropology, elemental analysis, cremains, inductively coupled plasma-optical emission spectroscopy, multivariate analysis

Burned human bones are physical evidence worthy of inquiry by anthropologists (1–5). Many questions are raised by the presence of burned bone, whether in an archaeological context (6–10), or in a forensic investigation (11–15). The product of modern cremation by professional crematories is the ultimate example of highly fragmented bone due to fire. Studies show that the choice of cremation is on the rise in the United States and Canada (16,17). A recent trend in the American cremation industry is the complete powderization of cremains. The powderization process is described by Warren and Schultz (18). Before this trend, bone fragments were large enough to be readily identified (19). For powderized cremains, a method is needed to address the fundamental question: are the cremains human or not?

The Tri-State Crematory Incident (Noble, GA; February 2001) highlighted the difficulties in identifying powderized cremains. It was discovered, that over a period of years, only a small portion of the bodies entrusted to the crematory were cremated. At least 339 bodies were illegally buried or concealed on the crematory property. Media reports informed families that the crematory had been putting nonhuman "filler," such as concrete and cement powder, in the urns, to make them appear legitimate. This caused affected families, and even other families not directly affected by the incident, to seek expert opinion about the contents of their urns. Some families brought their cremains for analysis to the Hamilton County Medical Examiner's Office (HCME), Chattanooga, TN. Conventional techniques for analyzing these cremains worked well for bone and tooth fragments, artifacts, and the weight of the set, but it could not be said with any scientific certainty whether the powderized portion was only human ash or not. The inability to visually distinguish human ash from contaminate powder in the Tri-State cremains was the impetus for this research project.

Most articles report case studies of a single individual rather than a hypothesis tested on the analysis of a group of cremains (13,19–23). Some population-level research has been directed at the total weight of cremains, particularly in an attempt to answer questions about the legitimacy of a set of cremains (24–26). Warren and Maples (25) showed that the cremains weight correlated better with cadaver length than cadaver weight. They interpreted this to mean that most of the ashes in a set of cremains represent the skeleton. Research has shown that most of the trace elements in humans are stored in the skeleton (27). For two decades, skeletal biologists have refined elemental and isotopic analysis of cremains (28–31). Inductively coupled plasma-optical emission spectroscopy (ICP-OES) has been used for the elemental analysis of bone ash, for example, Klepinger et al. (32) used it to investigate ancient diets. Another technique used to analyze cremains comes from Warren et al. (33). They performed a trace element analysis using particle-induced X-ray emission (PIXE), and were able to determine that the set of cremains was not legitimate. Although an effective technique, the instrument is costly and not widely available. ICP-OES detects and quantifies trace elements, is less expensive and more widely available, and has methodological acceptance in skeletal biology theory. ICP-OES was used in this study to test its efficacy on human cremains.

The premise of this project is that the chemical signature of a human body should still be identifiable by elemental analysis after cremation. For this study, the concentrations of 21 elements in samples of known human cremains and potential fillers (concrete

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and wood ash) were determined by ICP-OES. The elements included in the study were aluminum (Al), antimony (Sb), arsenic (As), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), potassium (K), selenium (Se), silver (Ag), strontium (Sr), thallium (Tl), and vanadium (V). These elements were chosen because standards for ICP-OES were readily available and they are representative of different groups of the periodic table. Following instrumental analysis, the data were statistically analyzed. Previous research by Bora et al. (34) used principal component and discriminant analysis with ICP-OES data from heroin samples. This cremains study used similar statistical tests to identify the elements most useful to distinguish between two sample groups: known human cremains and concrete. Questionable samples were analyzed using the same laboratory procedures and the probability of their group classification was computed with the discriminant function.

Materials and Methods

Samples

Table 1 details the sources of the samples used in this study. The 88 samples analyzed in this study were divided into three groups: known human cremains, known concrete, and questionable cremains. The known human cremains group ($n = 54$) was acquired by scientific donation to the HCME. Some were body donations, cremated under the direction of HCME at a single crematory. Others were samples taken from urns of cremains brought in by families who responded to a local newspaper article about the research project (35). In these cases, only donations accompanied by the *Certificate of Cremation* were accepted. Thirty-three of the known human samples were obtained from the University of Cincinnati Body Donation Program. The known concrete group ($n = 10$) consists of various brands of concrete, cement powder, and other construction bonding materials that mimic powderized ash. The questionable group ($n = 24$) consisted of six donations processed by the Tri-State Crematory, one canine (*Canis familiaris*), a sample of wood ashes, two individuals with aberrant Pb, Cu, and Mn levels, and 13 human/cement powder mixtures of varying ratios. These human/cement mixtures were prepared using ashes from three known humans and type I general purpose Portland cement powder. They were mixed in individual plastic containers and shaken by hand for 1 min to ensure homogeneity in those samples. The powderization process ensures homogenization of all the ashes that represent the skeleton, so removing a sample from anywhere in the urn is representative of the entire sample. Human-based research approval was obtained from the Institutional Review Board at the University of Tennessee at Chattanooga (IRB Application #04-128).

TABLE 1—*Samples used in this study.*

Sample Description	Status	N ($n = 88$)
HCME donations	Known	21
University of Cincinnati Body Donation Program (medical school cadavers)	Known	33
Canis familiaris (canine)	Questionable (Q)	1
Wood ashes (hardwood)	Questionable (Q)	1
Questionable human cremains*	Questionable (Q)	9
Human:concrete mixtures†	Questionable (Q)	13
First individual—90:10, 75:25, 40:60, 25:75		
Second individual—75:25, 60:40, 40;60, 10:90		
Third individual—90:10, 75:25, 60:40, 50:50, 25:75		
Concrete brands‡	Concrete	10

*Includes six Tri-State cases, two individuals with elevated Pb, Cu, and Mn levels due to indwelling projectiles, and a set from Oakland, CA, determined to be suspicious by its unusual color and complete powderization.

†Three known human donations from HCME and general purpose type 1 Portland cement were used to make these mixtures.

‡Various commercial brands of cement, concrete, and other construction bonding materials that mimic human ash.

TABLE 2—*Digestion procedure.*

Step	Heating Time (min)	Acid or Oxidant	Volume (mL)
1	10 at 100°C	HNO_3	10.0
2	Sample allowed to cool	N/A	0.0
3	5 at 100°C	HNO_3	5.0
4	Sample allowed to cool	N/A	0.0
5	Heat to a boil at 200°C	HNO_3	2.0
6	Sample allowed to cool	N/A	0.0
7	Heat to a boil at 200°C	H_2O_2	2.0

Materials, Sample Preparation and Instrumentation

The nitric acid used for the digestion was trace metal grade, purchased from Fisher Scientific (Fairlawn, NJ). The hydrogen peroxide (Fisher Scientific) was 30% (v/v) in water. The multi-element standard solutions (0.01, 0.1, 1.0, and 10.0 mg/L) for instrument calibration were prepared from 1000 mg/L single-element Assurance® Standards purchased from Spex Certiprep® (Metuchen, NJ). The elements analyzed include Al, Sb, As, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Li, Mn, Mo, Ni, K, Se, Ag, Sr, Tl, and V. All water used was distilled followed by deionization with a Barnsted® B-pure UltraPure™ Filtration System (Fisher Scientific) based on ion exchange technology.

All glassware used in the preparation process was cleaned in a 20% (v/v) nitric acid bath for at least 2 h and then rinsed three times with deionized, distilled water. To ensure precision, three 1.0 g subsamples of each sample were prepared by hot-plate digestion with nitric acid and hydrogen peroxide. The exact procedure is listed in Table 2. This process took *c*. 2 h per sample. Once digestion was complete, it was quantitatively transferred to a 100 mL volumetric flask and brought to volume. The samples were filtered with 0.22 µm nylon membrane filters (Fisher Scientific). All the digested samples were stored in 125 mL plastic bottles for a few days until analysis. A reagent blank was also prepared following the digestion procedure.

A Jobin-Yvon Ultima Inductively Coupled Plasma Optical Emission Spectrometer was used to analyze all samples. This is a sequential spectrometer with photomultiplier tube detection. Table 3 lists the instrument specifications and operating condi-

TABLE 3—*Specifications and operating conditions of ICP-OES.*

Monochromator	Czerny-Turner, 1.0 m
Grating	2400 grooves/mm, holographic
Detector	PMT
RF generator	1.5 kW at 40.68 MHz
Carrier gas flow rate	2.0 mL/min
Plasma gas flow rate	13 L/min
Plasma observation	Radial
Pump rate	2.0 mL/min

ICP-OES, Inductively Coupled Plasma-Optical Emission Spectroscopy.

TABLE 4—*Emission wavelengths and detection limits of 21 elements.*

Element	Wavelength (nm)	Detection Limit* (ppm)	Element	Wavelength (nm)	Detection Limit* (ppm)
Aluminum	308.215	5.98E − 03	Lithium	670.784	1.01E − 03
Antimony	206.833	3.05E − 02	Manganese	257.610	2.19E − 04
Arsenic	193.695	6.54E − 04	Molybdenum	202.030	5.52E − 01
Beryllium	313.042	1.35E − 04	Nickel	231.604	8.08E − 02
Boron	249.678	1.08E − 02	Potassium	766.490	3.67E − 02
Cadmium	226.502	5.67E − 04	Selenium	196.026	3.57E − 03
Chromium	276.716	8.49E − 04	Silver	328.068	4.99E − 03
Cobalt	228.616	2.32E − 04	Strontium	407.771	6.22E − 05
Copper	324.754	3.75E − 04	Thallium	190.864	7.57E − 03
Iron	259.940	5.55E − 03	Vanadium	292.402	6.93E − 04
Lead	220.353	3.81E − 03			

*Detection limit is calculated as ks_{bk}/m where k is the confidence factor (2), s_{bk} is the standard deviation of the blank measurement, and m is the slope of the calibration curve.

tions. The instrument used a cyclonic spray chamber with a cross-flow nebulizer for sample introduction. Nitrogen gas was used to purge the monochromator of air and dust. The analytical emission wavelengths and elemental detection limits are listed in Table 4. The instrument was calibrated against multielement standards. Digestions of three subsamples were made from each sample. Each subsample was analyzed three times and the outputs averaged. Mean values from the three subsamples were used to represent the concentration (mg/kg) of an element in that sample.

Statistics

As it was not known which elements would form a human signature, a variable cluster analysis of the known samples was used to identify the trace elements that formed a representative component. A principle component analysis was used to investigate further the trace element groupings. Lastly, based on the selected trace elements, a discriminant function was computed to assign the probability of group membership (cremains or concrete) to a questionable set of cremains. These data were statistically analyzed using SAS 9.1 for Windows (SAS Institute, Cary, NC).

Results

To determine what survives the cremation process, several laboratory techniques were used before ICP-OES. Organic extraction of the cremains by polar and nonpolar solvents and analysis by gas chromatography/mass spectrometry (GC/MS) did not detect any molecules. Additionally, the powdered cremains were analyzed by total-attenuated reflectance infrared spectroscopy (IR), but the resulting spectra did not indicate the presence of organic molecules. An elemental technique would be required for any type of definite analysis.

All samples were analyzed for 21 elements by ICP-OES. Only 16 elements with significant concentration values (Sb, As, B, Cd, Cr, Co, Cu, Pb, Li, Mn, Ni, Se, Ag, Sr, Tl, and V) were found. The concentrations of the remaining five elements (Al, Be, Fe, Mo, K) were found to be outside of the instrument calibration curves or below the detection limits. Investigation into the volatility of six elements (Sb, As, B, Cr, Mn, and Se) led to the removal of As and Se from the study. As and Se are volatile and their concentrations following cremation would be erratic due to the temperatures of the retort (1000°C or less) (36). Sb, B, Cr, and Mn are volatile as halides and halogens. In the oxygen-rich bone environment, these four elements are unlikely to exist in a reduced form. Oxides of these four elements have boiling points above the temperatures of the retort (37).

The mean within sample coefficient of variations (CV) are shown in Table 5. Most of the elements have relative errors less than 10%. The small variations are indicative of the precision of the methodology. The mean between sample standard deviations of the 14 elements for the concrete and cremains groups are listed in Table 6. The relatively large standard deviations represent the wide variability expected between samples due to the relatively small sample size. *t*-Tests show that the means of Sb, B, Co, Cu, Pb, Li, Sr, Tl, and V are significantly different between the two groups ($p < 0.001$) while the means of Cd, Cr, Mn, Ni, and Ag are not significantly different. Despite these differences, the large standard deviations and overlapping ranges imply that a single element cannot correctly classify the samples. Therefore, multivariate statistical methods were used for further analysis.

A goal of this study was to investigate the ability to separate questionable samples into one of the two known groups: cremains or concrete. All of the elements were not equally useful for discriminating between the two groups. The *t*-tests showed significant differences for some elements, but not others. The relatively small size of the concrete group makes interpretation of these means tests difficult. Additional analyses less dependent on normality assumptions were used to reduce the number of variables. Variable clustering and principal component analysis aided in the identification of the elements most useful for discriminating between these two groups. A disjoint variable clustering of the 14

TABLE 5—*Mean within sample coefficient of variation (CV = SD/mean × 100).*

Element	$N = 88$ (mean of CV)
Sb	5.6
B	7.4
Cd	3.9
Co	14.9
Cr	6.7
Cu	16.8
Pb	9.0
Li	6.4
Mn	8.9
Ni	20.2
Ag	16.6
Sr	4.0
Tl	4.0
V	6.0

CV, coefficient of variations.

TABLE 6—*Mean concentration (mg/kg) and standard deviation values for each element in the total sample set and each of the three groups: concrete, cremains, and questionable.*

Element	Concrete ($N = 10$)		Cremains ($N = 54$)	
	Mean	SD	Mean	SD
Sb*	24.7	22.9	17.4	7.6
B*	14.4	9.9	138.2	179.9
Cd	4.8	7.3	4.2	7.0
Co*	1.6	2.2	4.0	9.9
Cr	27.3	41.4	18.2	31.7
Cu*	10.6	18.2	92.2	187.2
Pb*	22.8	10.7	43.0	99.7
Li*	35.3	83.3	9.8	11.6
Mn	192.0	235.7	69.5	195.1
Ni	6.6	11.8	10.9	16.0
Ag	4.2	5.6	8.5	9.8
Sr*	265.9	213.2	127.2	91.9
Tl*	33.5	24.8	13.5	5.1
V*	22.7	27.8	5.1	5.3

*t-Tests show that the group means of these elements are different at the $p = 0.001$ level.

elements was performed. Cluster One contained seven elements: Sb, B, Li, Mn, Sr, Tl, and V. The remaining seven elements (Cd, Cr, Co, Cu, Pb, Ni, and Ag) formed Cluster Two.

The principal component analysis had five components with eigenvalues greater than one. These unrotated components accounted for 73% of the variability. The factor loadings for all five components are given in Table 7. The first three components are principally identified with unique groupings of elements. Component 1's largest loadings were on Sb, Cr, Li, Tl, and V. This component accounted for 28% of the total variability. As expected from the variable cluster analysis, Mn and Sr had positive loadings on Component 1. Component 2's largest loadings were on Cd and Pb and Component 3's on Co and Ni. Components 4 and 5 did not have large positive loadings on any element. Relatively large negative loadings were on Ag in Component 4 and Sr in Component 5, while B appeared with a positive loading in both components. Using the seven elements identified with the variable clustering, a linear discriminant function was computed for each group. Table 8 lists the coefficients of the discriminant function for each group. This function was used to classify the 24 samples in the questionable group. Average, minimum, and maximum discriminant function terms were computed to study the relative contribution of each element to the function. As shown in Table 9, Tl and V are the major terms of the average concrete classification functions while Sb and Tl are the largest terms of the cremains function. The highly variable elements, Li and Mn, tend to contribute less to the classification function. Table 10 shows the probability of group membership for these samples. For reliable classification into a group, the 0.90 probability level was used. Samples below 0.90 remained in the questionable group. The mixtures that were 50% or less human content were classified as concrete and those 90% human content were classified as cremains. The mixtures with 60% and 75% human content were not classified into either group and remained questionable. Wood ash classified into the concrete group, while the canine had a 0.91 probability of belonging to the cremains group.

Of the questionable samples, Q1, Q4, and Q7 had a probability more than 0.95 of classification as cremains. Q2, Q3, and Q6 also classified into the cremains group with a probability of 0.90 or more. Q5 remained questionable with a 0.80 probability in favor of cremains. The human with shotgun pellets (Q8) classified as cremains. However, the human with the copper-jacketed bullet (Q9) remained questionable with a 0.78 probability toward cremains.

Table 11 reorders the human/concrete mixtures by the source of human cremains and concentration. For each set of human cremains, the probability of belonging to the cremains group increases as the concentration of human cremains increases in the sample. Samples with 50% or less human content classified as concrete. Samples with 60–75% human content had a probability of 0.14–0.51 of belonging to the cremains group. Samples with 90% human content classified as cremains.

Discussion

The incident at the Tri-State Crematory and the powderization trend in the crematory industry has necessitated new standards for a chemical analysis of human cremains. The preliminary data reported in this pilot study indicate that it is possible to determine the legitimacy of cremains using elemental analysis by ICP-OES coupled with multivariate statistical analysis. However, because of the human/concrete mixture results, the meaning of terms like "legitimate" and "contaminated" needs to be addressed. The mixture results from Table 11 indicate that as the amount of human ash in the ratio decreases, the likelihood of a mixture being classified as concrete increases. This invites the question, where is the cut-off between legitimate and contaminated cremains? There may never be an answer to this question. It can be speculated

TABLE 7—*Factor pattern for principal component analysis of cremains and concrete groups.*

Element	Component 1	Component 2	Component 3	Component 4	Component 5
Sb	0.82	−0.16	0.03	0.06	0.23
B	−0.22	−0.04	−0.25	0.50	0.58
Cd	0.28	0.78	−0.23	−0.15	−0.10
Co	0.08	0.20	0.79	0.36	−0.07
Cr	0.71	0.57	−0.23	−0.05	0.06
Cu	0.01	0.42	0.30	0.21	−0.06
Pb	0.32	0.85	−0.26	−0.08	−0.02
Li	0.80	−0.18	−0.09	0.28	0.21
Mn	0.51	−0.31	0.17	−0.36	0.08
Ni	0.30	0.41	0.69	0.05	0.08
Ag	−0.02	−0.02	0.34	−0.70	0.45
Sr	0.39	−0.30	0.01	0.00	−0.60
Tl	0.86	−0.28	−0.08	0.07	−0.02
V	0.79	−0.33	0.03	−0.01	−0.06
% variance	28%	18%	11%	8%	7%

TABLE 8—*Linear discriminant functions for cremains and concrete groups with Sb, B, Li, Mn, Sr, Tl, and V.*

Variable	Seven Elements Sb, B, Li, Mn, Sr, Tl, and V	
	Concrete	Cremains
Constant	−12.091	−4.780
Sb	0.171	0.282
B	0.005	0.009
Li	−0.134	−0.095
Mn	−0.004	−0.003
Sr	0.019	0.013
Tl	0.557	0.244
V	−0.092	−0.144

that the ratio to which someone would contaminate a set of human ashes could be anywhere from a small amount to complete replacement of ash with filler. The entire process of modern cremation introduces some contamination from the retort. This small amount of contamination was accepted as part of the overall elemental signature, making this a natural experiment versus a controlled experiment. To quantify how much nonhuman material is introduced by the whole process would require controlled laboratory conditions and would not be reflective of real-world cremation processes. Deciding whether any amount of contamination is significant is a legal matter that depends on the crematory's intent, which is not a matter for forensic scientists, but rather for the courts. Conventional cremains analysis techniques should still be used, but the method introduced here could detect a large replacement (>50%) of human ash in the urn with other powders that mimic human ash in weight, texture, and color.

As shown in Table 10, the technique was unable to distinguish cremains at the genus level (*Homo* vs. *Canis*), but the skeleton of *Canis familiaris* was not expected to differ drastically in chemistry from the human skeleton. The wood ashes were introduced as a questionable sample because they are visually comparable with powderized human ash. As might be expected, the wood ashes strongly classified with the concrete group and the canine strongly classified with the cremains group.

Visual analysis of Q1 initially classified it as questionable because of its total powderization and yellowish color not normally seen in the "battleship grays" of most cremains. After visually examining the Cincinnati medical school cremains, it was apparent that human ashes vary wider in color than initially thought. Following elemental analysis and discriminant classification, Q1 classified as cremains. This case illustrates that a visual examination of the powderized portion is subjective and unreliable, and that a chemical method is more objective.

TABLE 9—*Mean, min, and max terms of linear discriminant functions for cremains and concrete groups with Sb, B, Li, Mn, Sr, Tl, and V.*

Variable	Seven Elements Sb, B, Li, Mn, Sr, Tl, and V					
	Concrete			Cremains		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Constant	−12.09	−12.09	−12.09	−4.78	−4.78	−4.78
Sb	4.22	1.18	14.17	4.91	2.45	14.56
B	0.07	0.00	0.17	1.20	0.13	7.91
Li	−4.71	−0.28	−36.01	−0.94	−0.13	−6.82
Mn	0.65	0.01	2.47	−0.19	0.00	−2.83
Sr	5.00	1.69	13.12	1.65	0.74	7.68
Tl	18.63	7.50	50.16	3.30	1.94	7.17
V	12.66	1.03	43.89	1.24	0.26	8.98
Total	24.43	−0.97	75.88	6.41	0.60	31.88

TABLE 10—*Posterior probability of group membership using linear discriminant functions from Table 8, ordered by ratios.*

$N = 24$ Sample	Seven Elements Sb, B, Li, Mn, Sr, Tl, and V		
	Concrete	Cremains	Group
10:90-2*	1.00	0.00	Concrete
25:75-1	1.00	0.00	Concrete
25:75-3	1.00	0.00	Concrete
40:60-1	1.00	0.00	Concrete
40:60-2	1.00	0.00	Concrete
50:50-3	0.99	0.01	Concrete
60:40-2	0.86	0.14	Questionable
60:40-3	0.65	0.35	Questionable
75:25-1	0.56	0.44	Questionable
75:25-2	0.18	0.82	Questionable
75:25-3	0.49	0.51	Questionable
90:10-1	0.09	0.91	Cremains
90:10-3	0.01	0.99	Cremains
Wood ash	1.00	0.00	Concrete
Canine	0.09	0.91	Cremains
Q1	0.02	0.98	Cremains
Q2	0.07	0.93	Cremains
Q3	0.08	0.92	Cremains
Q4	0.04	0.96	Cremains
Q5	0.20	0.80	Questionable
Q6	0.09	0.91	Cremains
Q7	0.01	0.99	Cremains
Q8†	0.01	0.99	Cremains
Q9‡	0.22	0.78	Questionable

*The ratio represents human/concrete by mass. The number indicates the source of the known human cremains from which they were mixed.
†Individual with indwelling shotgun pellets.
‡Individual with indwelling copper-jacketed bullet.

Most people with knowledge of the Tri-State Crematory Incident would expect the questionable Tri-State cremains (Q2, Q3, Q4, Q5, Q6, and Q7) to be classified as concrete. Of these, only Q5 was not classified as cremains. The family of Q5 had taken the cremains for analysis to the Georgia Bureau of Investigation, who told the family that the contents of the urn were human. Unconvinced, the family stored the ashes outdoors in a shed, where they were subjected to rain and standing water until they were brought to the HCME for a second analysis. The cremains weighed 1.86 kg when brought in wet and 1.36 kg after drying. It is possible that trace elements were introduced into the cremains via rainwater

TABLE 11—*Posterior probabilities of mixtures, ordered by source of known human cremains.*

$N = 13$ Sample	Seven Elements Sb, B, Li, Mn, Sr, Tl, and V		
	Concrete	Cremains	Group
25:75-1	1.00	0.00	Concrete
40:60-1	1.00	0.00	Concrete
75:25-1	0.56	0.44	Questionable
90:10-1	0.09	0.91	Cremains
10:90-2	1.00	0.00	Concrete
40:60-2	1.00	0.00	Concrete
60:40-2	0.86	0.14	Questionable
75:25-2	0.18	0.82	Questionable
25:75-3	1.00	0.00	Concrete
50:50-3	0.99	0.01	Concrete
60:40-3	0.65	0.35	Questionable
75:25-3	0.49	0.51	Questionable
90:10-3	0.01	0.99	Cremains

that altered the cremains at the elemental level (a form of diagenesis). The remaining five sets were classified as cremains, indicating that not every set of cremains returned by the Tri-State Crematory was filled with nonhuman material.

Both Q8 and Q9 are known human cremains, yet Q9 did not definitely classify as cremains. These two individuals were both shot earlier in life and the bullets remained in their bodies. Q8 had been shot with lead buckshot and exhibited the second highest level of Pb, but Cu and Mn levels were consistent with the rest of the known cremains. The other individual (Q9) had been shot with a copper-jacketed bullet and exhibited the highest Pb level known and very high Cu and Mn levels. Because of their history, they were placed into the questionable group to see if this technique would "ignore" aberrant levels of elements and consider the overall trace element signature. The high level of Mn in Q9 lowered its probability of classification as cremains to 0.78 because Mn is one of the elements used to define the discriminant function. None of the other constituents of lead buckshot (such as Sb, used as a hardener in the manufacture of such ammunition) were noticeably elevated in sample Q8. The amount of Sb detected by ICP-OES in this individual (29.4 mg/kg) was above the mean of the known human cremains in the study (17.4 mg/kg, $n = 54$), but was not aberrantly higher like Pb. The best answer at this time is that Sb is already at a trace amount in the prefired projectile, and therefore its subsequent migration through scar tissue over time added very little to the individual's total body burden. The results of these questionable samples emphasize the need for larger sample sizes to better define concentration ranges used to develop multielement discriminant functions.

This pilot study has identified several methodological issues that should be incorporated into future research. First, it was only after ICP testing was concluded that the researchers became aware that a sample of bone ash certified by the National Institute of Standards and Technology (NIST, SRM 1400) was available. Discussion with NIST personnel about this sample revealed that it is not human bone ash; nonetheless, it still would provide validation of the acid digestion procedure.

Of the 21 elements tested, 16 proved to be within detectable limits, and only seven of those were selected for their predictive value. The underlying reason for why these seven elements provide a classification function is not understood at this time, but it is most likely due to a complex interaction between an individual's culture and physiology. In many communities around America, socioeconomic status is correlated with residence patterns around heavy industry with chemical output that migrates into drinking water, food, and the air. Future studies should attempt to control for the residence patterns and specific geographical and environmental exposures of cremated individuals, as some elements accumulate in bone tissues at a rate faster than they are excreted (e.g., Pb).

Another difficulty encountered in this study was the presence of elemental concentration outliers in single individuals. While interviews with the donor families explained the elevated Pb, Cu, and Mn levels in the two individuals with indwelling projectiles, other interviews did not offer useful explanations. For example, one individual exhibited an Ag level substantially higher than all other known, but interviews with this family revealed no specific exposure except that the person had lived in heavily industrialized cities. Vague personal information like this indicates that a more structured questionnaire of donors is needed to document the demographic, medical, occupational, and socioeconomic history of the decedent so that the significance of extreme values can be understood.

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